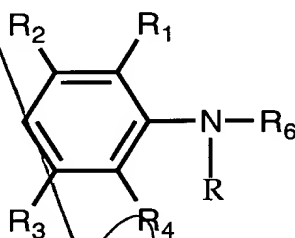


WE CLAIM:

1. An analytical element for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

- (a) an arylacylamidase enzyme;
- (b) an oxidizing agent selected from the group consisting of an enzyme and a ferricyanide capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and
- (c) a water-soluble, color-forming, coupling agent of the general structure:



wherein R is a water-solubilizing group selected from $-(CH_2)_nX$ where n is 1 to 5 and X is $-SO_3M$ where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium (NH_4^+) cation, or $-N(R_7)_3^+Z^-$ where each R_7 is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is $(-OCH_2CH_2)_yOH$ where y is 2 to 5;

R_1 and R_6 are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

R_2 , R_3 , and R_4 are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

2. An analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

8. The element of ~~claim 2~~ further containing maleimide.

9. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support and in fluid contact:

(a) one or more layers having therein an arylacylamidase enzyme; a ferricyanide capable of oxidatively coupling paraaminophenol to a color-forming coupler to form a color compound; and a water-soluble, color-forming coupling agent as defined in claim 1; and

(b) a porous spreading layer.

10. The element of claim 3 wherein the ferricyanide is a ferricyanide salt of an alkali metal.

11. The element of claim 3 wherein the coupling agent is 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline.

12. The element of claim 3 further containing a buffer for maintaining the pH of the element in a range of about 6.5 to 8.5.

13. The element of claim 3 further containing maleimide.

14. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support a first and second reagent layer wherein:

the first reagent layer having therein 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline;

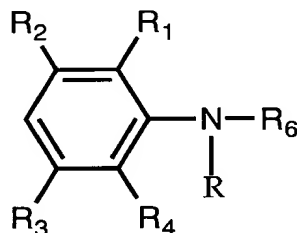
the second reagent layer having therein ascorbic acid oxidase or a ferricyanide salt and arylacylamidase; and
a porous spreading layer.

15. The element of claim 14 further containing maleimide.

16. The element of claim 15 wherein the maleimide is in the spreading layer.

- (a) an arylacylamidase enzyme;
(b) an oxidizing enzyme capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and

5 (c) a water-soluble, color-forming, coupling agent of the general structure:



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wherein R is a water-solubilizing group selected from $-(CH_2)_nX$ where n is 1 to 5 and X is $-SO_3M$ where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium (NH_4^+) cation, or $-N(R_7)_3^+Z^-$ where each R₇ is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is $(-OCH_2CH_2)_yOH$ where y is 2 to 5;

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R₁ and R₆ are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

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R₂, R₃, and R₄ are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

3. An analytical of element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

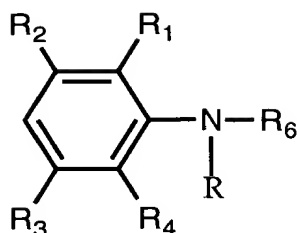
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(a) an arylacylamidase enzyme;

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(b) a ferricyanide capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and

(c) a water-soluble, color-forming, coupling agent of the general structure:



wherein R is a water-solubilizing group selected from $-(CH_2)_nX$ where n is 1 to 5 and X is $-SO_3M$ where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium (NH_4^+) cation, or $-N(R_7)_3^+Z^-$ where each R₇ is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is $(-OCH_2CH_2)_yOH$ where y is 2 to 5;

R₁ and R₆ are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

R₂, R₃, and R₄ are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

4. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support and in fluid contact:

(a) one or more layers having therein an ~~an~~ ~~acyl~~amidase enzyme; an oxidizing enzyme capable of oxidatively coupling paraaminophenol to a color-forming coupler to form a color compound; and a water-soluble, color-forming coupling agent as defined in claim 1; and

(b) a porous spreading layer.

5. The element of claim 2 wherein the oxidizing enzyme is selected from the group consisting of ascorbic acid oxidase, lactase, and tyrosinase.

6. The element of claim 2 wherein the coupling agent is 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline.

7. The element of claim 2, further containing a buffer for maintaining the pH of the element in a range of about 6.5 to about 8.5.

17. A method for determining acetaminophen in an aqueous liquid comprising the steps of:

- a. contacting a sample of the aqueous liquid with the analytical element of claim 1, 2, 3, or 14; and
- b. correlating the amount of color compound formed to the concentration of acetaminophen in the fluid.